Mol Breeding
DOI 10.1007/s11032-006-9034-1

QTL analyses of fiber components and crude protein in an annual × perennial ryegrass interspecific hybrid population

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Received: 11 May 2006 / Accepted: 8 July 2006

Abstract Annual (*Lolium multiflorum* Lam.) and perennial (*Lolium perenne* L.) ryegrasses are two important forage and turfgrass species. Improving the digestibility of forage by decreasing fiber content is a major goal in forage crop breeding programs. An annual × perennial ryegrass interspecific hybrid population was used to map quantitative trait loci (QTLs) for fiber components, neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL), and crude protein (CP). Samples were harvested three times in August and September 2003 and August 2004, respectively. Simple interval mapping was used to detect QTLs from both the male and female parental maps previously developed for the population. Fiber components were all correlated positively with each other and were negatively correlated with CP. The largest correlations were between NDF and ADF with r = 0.86, 0.72, and 0.82 for each of the three harvests. All four traits showed intermediate broad-sense heritability values ranging from 0.35 to 0.72. A total of 63 QTLs were detected for the four traits measured over the three harvests from both the female and male maps. Coincident QTLs were detected on linkage groups (LGs) 2, 6, and 7 for NDF, LGs 1, 2, and 7 for ADF, LGs 6 and 7 for ADL, and LG 2 for CP, respectively. Coincident QTLs were also detected on LGs 2, 6, and 7 for NDF and ADF, providing evidence of the genetic basis of the observed high level of phenotypic correlation. The QTLs on LGs 2, 6, and possibly 7 for fiber components were co-located on the same LG as several lignin biosynthetic genes from perennial ryegrass.

Keywords Forage quality · *Lolium multiflorum* Lam. · *Lolium perenne* L. · Quantitative trait loci

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Abbreviations

ADF	Acid detergent fiber
ADL	Acid detergent lignin
CP	Crude protein
LG	Linkage group
NDF	Neutral detergent fiber
QTLs	Quantitative trait loci

Introduction

Perennial ryegrass (*Lolium perenne* L.) and annual ryegrass (*Lolium multiflorum* Lam.) are used widely as both forage and turfgrasses. Improving digestibility is an important goal in forage ryegrass breeding programs because high digestibility maximizes forage intake and improves efficiency of nutrient conversion by animals. The composition and content of cell walls are key factors affecting herbage digestibility. Cell walls are predominantly composed of cellulose, hemicellulose, and lignin. The presence of lignin limits the digestion of intact cell walls (Moore and Hatfield 1994; Cardinal et al. 2003). Cell-wall digestibility is negatively correlated with cell-wall lignin and fiber concentrations (Lundvall et al. 1994; Wolf et al. 1993). Thus, improving cell-wall digestibility could be achieved by decreasing fiber components.

Forage quality can be evaluated directly by feeding experiments, but this method is costly and not possible with low quantities of breeding materials (Lübberstedt et al. 1997). Indirect methods of assessment include in vitro digestibility with rumen liquor (Tilly and Terry 1963; Menke et al. 1979), enzymatic digestion (De Boever et al. 1986) and chemical analysis of cellular components (Van Soest 1963). However, chemical analyses for forage quality are slow and laborious, especially for multiple measurements of nutritive value of larger samples. Near-infrared reflectance spectroscopy (NIRS) offers an inexpensive, rapid, and accurate technique to evaluate forage-quality traits for breeding materials, although some chemical analyses are required for calibration. NIRS has been used as a routine method to evaluate forage quality in many forage species (Roberts et al. 2004). Use of NIRS allows measurements of various forge quality traits to be obtained simultaneously.

The conventional breeding method that exploits substantial genetic variation and then selects desirable traits remains the most successful approach to improve quality traits of herbage in ryegrasses. Phenotypic selection for high digestibility has been successful in forage crop species (Casler 2001). However, measurements for phenotypic traits like fiber, lignin and protein are still time consuming and expensive. With the rapid development of dense linkage maps based on molecular markers such as amplified fragment length polymorphism (AFLP), simple sequence repeat (SSR), rapid amplified polymorphism of DNA (RAPD), and restricted fragment length polymorphism (RFLP), it is possible to detect quantitative trait loci (QTLs) responsible for quantitative variation and use the information for marker-assisted selection (MAS) to improve selection efficiency. MAS should be superior to conventional phenotypic selection for those traits that have low heritabilities and for which the phenotypes are difficult or expensive to evaluate. Molecular marker-based genetic maps of perennial ryegrass have been constructed by using the p150/112 reference genetic mapping population (Jones et al. 2002a, b), and were used to map QTLs that were related to herbage quality traits such as in vivo dry matter digestibility (IVVDMD), neutral detergent fiber (NDF), estimated metabolizable energy (EstME), and crude protein (CP) (Cogan et al. 2005). A total of 13 QTLs for NDF, eight QTLs for EstME, and seven QTLs for CP were observed from six different sampling experiments varying by developmental stage, location or year. A coincident QTL for NDF was located on LG 7 and was close to the locations of the lignin biosynthesis genes *Lp*CCR1 (cinnamoyl CoA-reductase), *Lp*OMT1 (caffeic acid-*O*-methyltransferase) and *Lp*CAD2 (cinnamoyl alcohol dehydrogenase) (Heath et al. 1998; Lynch et al. 2002; McInnes et al. 2002; Cogan et al. 2005). The QTLs affecting fiber components and CP concentration have also been examined in forage maize (Lübberstedt et al. 1997, 1998; Méchin et al. 2001). Cardinal et al.

(2003) identified a total of 65 QTLs that were associated with fiber and lignin content in maize.

Warnke et al. (2004) constructed female and male genetic linkage maps from an interspecific annual × perennial ryegrass interspecific mapping population based on 81 RAPD, 235 AFLP, 16 RFLP, 106 SSR, 2 isozymes, and 2 morphological markers. The genetic maps were enhanced by 120 additional RFLP markers from barely (*Hordeum vulgare* L.), oat (*Avena sativa* L.), and rice (*Oryza sativa* L.). Comparative mapping using anchor markers has allowed the alignment of the ryegrass genetic maps with those of Triticeae, oat, and rice and revealed substantial conserved synteny between the genome of the ryegrass and those of the Triticeae species, oat and rice (Jones et al. 2002b; Sim et al. 2005). The enhanced genetic maps provide the basis for mapping the QTLs responsible for phenotypic variations that exist in the mapping population. Four QTLs that were associated with resistance to the fungal disease of gray leaf spot have been identified by using the enhanced genetic maps in this population (Curley et al. 2005). The objective of the study reported here was to map QTLs that affect fiber components and CP of ryegrass herbage in the interspecific mapping population. The specific traits measured were NDF, acid detergent fiber (ADF), acid detergent lignin (ADL), and CP. The QTLs observed in varying developmental stage or year was compared and the heritabilities for each trait and the relationships between traits were examined as well. The identification of genomic regions that control the fiber components and CP content would improve our genetic understanding of these traits and provide the basis for MAS for ryegrass breeding and for identification of genes involved in ryegrass cell-wall digestibility.

Materials and methods

Plant materials

An annual ryegrass plant from the cultivar 'Floregon' was crossed to a perennial ryegrass plant from the cultivar 'Manhattan' to create the 1st F_1 population. At the same time, a second F_1 population was created in a similar manner but with two different parental plants each selected from *L. multiflorum* and *L. perenne* L., respectively. From the 1st F_1 population, one plant named MFA was chosen to cross with another plant named MFB that was chosen from the 2nd F_1 population to develop a three-generation population (a pseudo F_2 population). All of the seed used to develop the progeny population was obtained from the plant MFA, making MFA the female parent and MFB the male parent. The progeny population used for mapping includes 152 individuals. More details on the population development were described by Warnke et al. (2004)

Phenotypic data analysis

The mapping population has been maintained in a research greenhouse at 20–21°C at Iowa State University (Ames, IA, USA). Twelve single-tiller ramets were propagated for each of the 152 genotypes and their parents and were maintained in the same greenhouse. On 15 May 2003, four ramets of each genotype for each replication were transplanted to the field in an alpha-lattice design with three replications at the Iowa State University Horticulture Research Station near Gilbert, IA. Each replication included 26 blocks and each block included six entries. Spacing between ramets within each genotype was 30 cm. Each genotype was separated by a 60 cm space in between. Plants were mowed at 6.4 cm above the soil surface on 17 July 2003 to encourage regrowth. Each plant was then hand-harvested by a sharp sickle. The cutting height was about 6.4 cm. The first harvest was taken on 14 August 2003 and the second on 22 September 2003. The experiment was repeated at a different site within the same research station in 2004 except that in 2004, the plants were transplanted to the field on 3 June and mowed on 22 July and harvested on 21 August 2004. After each harvest, samples were dried for 5 days at 60°C in a forced-air dryer.

Dried plants were first ground with a Wiley mill and then reground with a UDY cyclone mill (Cyclone Mill, UDY Mfg., Fort Collins, CO, USA) to pass a 1-mm mesh screen. Ground samples were analyzed by near-infrared reflectance spectroscopy (NIRS). A Pacific Scientific

6250 scanning monochromator was used with wavelengths ranging from 1,100 nm to 2,500 nm spaced 4 nm apart to determine reflectance (NIRS Systems, Silver Spring, MD, USA). Separate calibration sets were selected from 2003 and 2004 harvests. Fifty and forty calibration samples were selected for 2003 and 2004 harvest calibration sets, respectively. The calibration sets represented the range of *H*-value for the entire sample set (Shenk and Westerhaus 1991a). One gram of each sample was dried for at least 2 h at 100°C to obtain the dry matter percentage. Samples from each calibration set were analyzed in triplet to determine NDF, ADF, and ash-free ADL. An ANKOM 200 Fiber Analyzer (ANKOM Technology Corp., Fairport, NY, USA) was used to determine NDF and ADF for the calibration sets (Vogel et al. 1999). Ash and ADL concentration were determined for the calibration set based on Van Soest et al. (1991). Nitrogen concentration was determined by combustion (LECO system CHN-2000) (Gavlak et al. 1994), and CP concentration was calculated by multiplying nitrogen by 6.25. All data were reported on a dry matter basis.

NIRS prediction equations were developed separately for NDF, ADF, ADL and CP for 2003 and 2004 harvests using modified partial least squares regression (Shenk and Westerhaus 1991b) with the Infrasoft International NIRS ver. 3.0 software program (ISI, Port Matilda, MA, USA.). Coefficients of determination (R^2), standard errors of the calibration (SEC) and the cross validation (SECV) of prediction equations for the 2003 harvest calibration set were 0.99, 0.38, and 1.18 for NDF; 0.99, 0.20, and 0.66 for ADF; 0.88, 0.14, and 0.19 for ADL; and 0.99, 0.04, and 0.10 for CP, respectively. The R^2, SEC, and SECV of prediction equations for the 2004 harvest calibration set were 0.98, 0.57, and1.07 for NDF; 0.93, 0.52, and 0.68 for ADF; 0.87, 0.12, and 0.16 for ADL; and 0.98, 0.06, and 0.11 for CP, respectively. NDF, ADF, ADL and CP values were predicted for all samples by using the prediction equations developed. The predicted values were used for further data analysis.

Analysis of variance of the phenotypic data was performed by the GLM procedure of the Statistical Analysis System (2000) (version 8.0, SAS Institute Inc., Cary, NC, USA). The MIXED procedure was used to estimate variance components with all effects in the model considered random. Broad-sense heritabilities (H) were calculated as $H = \sigma_g^2/(\sigma_g^2 + \sigma\ ^2_e/r)$, where σ_g^2 is the genetic variance and σ_e^2 is the error variance divided by r, the number of replications for each genotype. Pearson correlation coefficients were used to evaluate correlations for pair-wise phenotypic traits by using the CORR procedure of SAS. The correlations were calculated on a genotype-mean basis and calculated separately for each year.

QTL analysis

The genetic markers developed by Warnke et al. (2004) including RAPD, AFLP, RFLP, SSR, isozyme, and morphological markers were combined with additional RFLP marker data generated recently (Sim et al. 2005) to produce a composite marker dataset for QTL analysis of the present study. Two parental maps, MFA and MFB, were constructed by using the CP (cross pollination) option in Joinmap® (Van Ooijen and Voorrips 2001). Interval mapping analysis was employed to detect QTLs by using MapQTL 4.0 (Van Ooijen et al. 2002). Mean trait values for each harvest were used for detecting putative QTLs in both female parental (MFA) and male parental (MFB) maps. A logarithm of odds (LOD) score of 3.0 was chosen as the threshold for declaring putative QTLs. QTL positions were determined by the maximum LOD score. A confidence interval for the location of a QTL on the genetic map was determined by subtracting one LOD unit on each side from the maximum LOD position.

Results

Phenotypic distributions

A wide range of variations were observed for all four traits measured for the three harvests in the mapping population (Table 1; Fig. 1). The distribution of parental phenotypic means varied for each trait but all fell within the range of progeny individuals. The progenies showed near

Table 1 Mean and range of NDF, ADF, ADL, and CP for the parents and progenies in the annual × perennial ryegrass mapping population and the broad sense heritabilities for NDF, ADF, ADL, and CP at three harvests

Trait (g kg^{-1} DM)	Parents[a]		Progenies		Heritability (*H*)
	MFA	MFB	Mean	Range	
First harvest (Aug-03)[b]					
NDF	440.9	490.7	461.7	412.5–557.2	0.61
ADF	216.7	247.6	226.7	196.3–269.6	0.52
ADL	15.6	22.8	17.7	13–25.9	0.72
CP	244.4	248.8	241.2	191.9–273.4	0.62
Second harvest (Sep-03)					
NDF	409.9	423.5	436.1	371.9–514.8	0.50
ADF	203.3	206.6	212.1	178.9–245.8	0.35
ADL	15.7	16.9	16.2	12.3–21.3	0.46
CP	218.8	224.4	203.5	157.1–258.8	0.60
Third harvest (Aug-04)					
NDF	446.5	465.8	441.8	365.1–489.8	0.53
ADF	221.1	243.6	221.8	181.2–250.5	0.62
ADL	15	17.6	15.6	11.3–21.1	0.68
CP	227.5	231.3	240.4	200.8–308.1	0.65

[a] MFA and MFB were female and male parents for the annual × perennial ryegrass mapping population, respectively

[b] The first harvest was taken in Aug. 2003, and the second was in Sep. 2003, and the third was in Aug. 2004

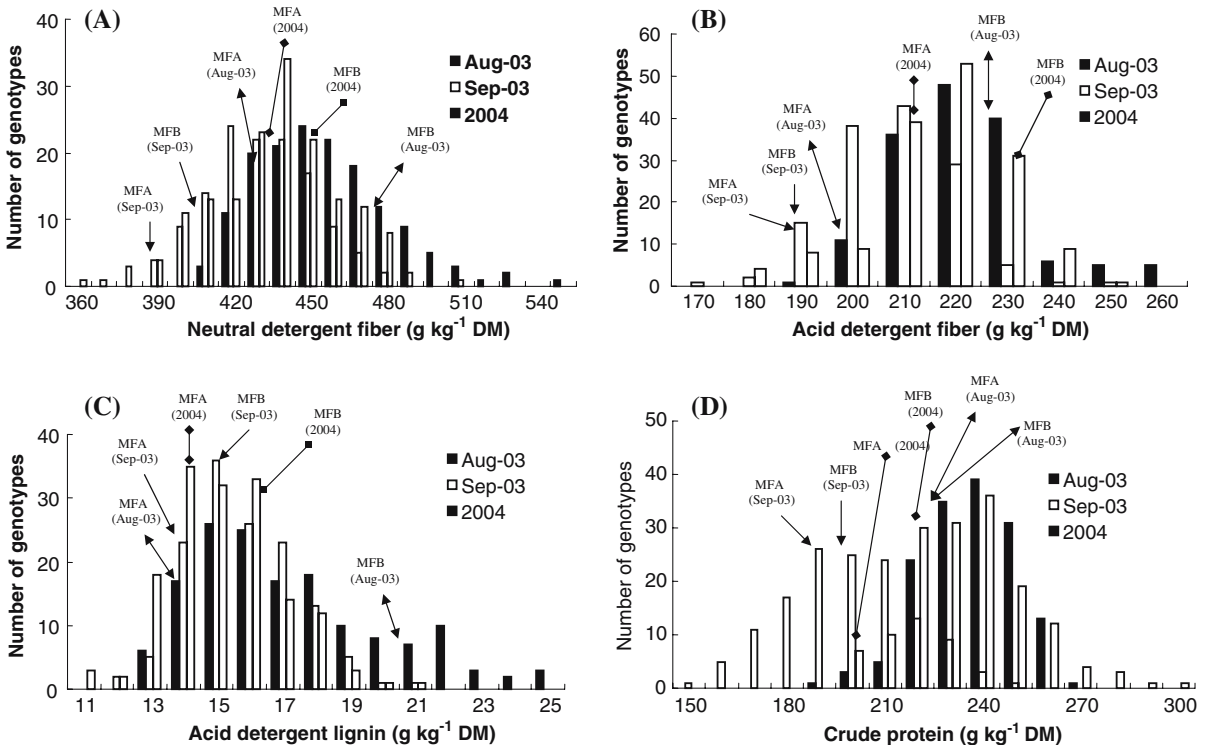

Fig. 1 Phenotypic distribution for NDF (**A**), ADF (**B**), ADL (**C**), and CP (**D**) for three harvests. The first, second, and third harvests were taken in Aug., Sep. 2003, and Aug. 2004, respectively. The means of the parents (MFA and MFB) are indicated by arrows

normal distributions for all traits. Most trait means of the progenies except for ADL were the highest in the August harvest of 2003, and were the lowest in the 2003 September harvest, and intermediate for the 2004 harvest.

Phenotypic correlations and heritabilities

The broad-sense heritabilities values ranged from 0.35 for ADF at the second harvest taken in September 2003 to 0.72 for ADL at the first harvest taken in August 2003 (Table 1). As shown in Table 2, phenotypic correlations between all traits were detected within each harvest except for between ADL and CP in the August and September harvests of 2003. The three largest phenotypic correlations were between NDF1 and ADF1 ($r = 0.86$), NDF3 and ADF3 ($r = 0.82$), and NDF2 and ADF2 ($r = 0.72$). All fiber components (NDF, ADF, and ADL) were positively correlated with each other. NDF and ADF were correlated negatively with CP. Correlations between NDF and ADF were higher than correlations between NDF and ADL, and ADF and ADL.

QTL analysis

A total of 63 QTLs were detected for the four traits across three harvests from both the female and male parental maps (Tables 3, 4; Figs. 2, 3). Twenty-six of them were from the female MFA map and 37 were from the male MFB map. The QTLs identified were distributed on every LG except for the LG 3 on the male map (Figs. 2, 3). Variable numbers of QTLs were found from different harvests. The highest number of QTLs was detected in the 2004 harvest, with 11 QTLs on the MFA map and 14 QTLs on the MFB map. The least number of QTLs detected was in the August harvest of 2003, with six QTLs on the MFA map and 11 QTLs on the MFB map. No QTLs related to ADL was found on the MFA map in the 2003 September and 2004 harvests. Detailed descriptions of the QTLs for each trait are provided below.

NDF

A total of 19 QTLs were identified for NDF across the three harvests (Tables 3, 4; Figs. 2, 3), of which 9 were from the female and 10 from the male map. The QTLs were distributed on LGs 1, 2, 3, 5, 6, and 7 on the female map, and LGs 1, 2, 6 and 7 on the male map, and explained phenotypic variation ranging from 12.4% to 41.5%. Coincident QTLs for NDF were identified on LG 7 on the female map from all three harvests, and on LGs 2, 6 and 7 on the male map from the 2003 August and 2004 harvests, the August and September harvests of 2003, and the 2003 August and 2004 harvests, respectively. QTLs for NDF were found on LGs 1, 2, 6 and 7 on both female and male maps. Single individual QTLs for NDF was detected on LGs 3 and 5 on the female map from the 2004 harvest.

ADF

A total of 20 QTLs for ADF were found for the three harvests from the female and male maps. Ten QTLs were detected on the female and male map, respectively (Tables 3, 4; Figs. 2, 3). The QTLs were mapped on LGs 1, 3, and 7 on the female map and LGs 1, 2, 6 and 7 on the male map and accounted for 10.1%–47.7% of the phenotypic variation. Consistent QTLs for ADF were found on LG1 from the 2003 and 2004 harvests and LG7 from all the three harvests on the female map. Similarly, coincident QTLs were found for ADF on LGs 1, 2 and 7 on the male map from two of the three harvests. Consistent QTLs were found on LG 1 and 7 from both female and male maps. Two single individual QTLs were mapped on LG 3 and LG 6 on the female and male map, respectively.

ADL

A total of ten QTLs were detected for ADL for three harvests from both female and male maps (Tables 3, 4; Figs. 2, 3). Nine of them were observed on the male map and only one was on the female map. The QTLs identified on the female map was located on LG 7 and explained 13.0% of the phenotypic variation and the QTLs identified on the male map were mapped on LGs 2, 5, 6 and 7 and accounted for phenotypic variation ranging from 11.7% to 45%. Coincident QTLs were found on LGs 6 and 7 from the three harvests and

Table 2 Pearson correlation coefficients between genotypic means for NDF, ADF, ADL, and CP measured at three harvests in the annual × perennial ryegrass population

	NDF1[a]	ADF1	ADL1	CP1	NDF2[b]	ADF2	ADL2	CP2	NDF3[c]	ADF3	ADL3
ADF1	0.864***										
ADL1	0.740***	0.647***									
CP1	–0.485***	–0.455***	–0.085ns								
NDF2	0.243***	0.172**	0.127*	–0.042ns							
ADF2	0.051ns	0.113ns	–0.092ns	–0.031ns	0.717***						
ADL2	0.264***	0.263***	0.454***	0.101ns	0.558***	0.268***					
CP2	0.163**	0.167***	0.220***	0.213***	–0.338***	–0.391***	0.191ns				
NDF3	0.155**	0.160**	0.044ns	–0.039ns	0.184**	0.109ns	0.125*	0.096ns			
ADF3	0.223***	0.262***	0.094ns	–0.039ns	0.112*	0.072ns	0.115*	0.146**	0.815***		
ADL3	0.127ns	0.135*	0.252***	0.067ns	0.027ns	–0.080ns	0.226***	0.182**	0.581***	0.454***	
CP3	–0.032ns	–0.026ns	0.127ns	0.147**	–0.034ns	–0.061ns	0.026ns	0.202***	–0.600***	–0.683***	–0.146**

[a] One represents the first harvest that was taken in Aug. 2003

[b] Two represents the second harvest taken in Sep. 2003

[c] Three represents the third harvest taken in Aug. 2004

The number of genotypes used for calculation of correlation coefficients was $n = 152$

ns, *, **, and *** indicate non-significant, or significant at the 0.05, 0.01, and 0.001 probability level, respectively

the two harvests from 2003, respectively, on the male map, and the QTLs located on LG7 was common on both the female and male maps.

CP

A total of 14 QTLs for CP were identified from both female and male maps for the three harvests (Tables 3, 4; Figs. 2, 3). Six of which were from the female map and eight from the male map. The QTLs on the female map were located on LGs 1, 2, 4 and 5 and the QTLs on the male map were mapped to LGs 1, 2, 4, 5 and 7. One coincident QTL was found on LG 2 from the September 2003 and 2004 harvests on both the female and the male maps, and explained more than 15% of the phenotypic variation. Single individual QTLs were detected on LGs 1,2, 4, 5 on the female map and LGs 1, 2, 4, 5, and 7 on the male map from a single harvest.

Discussion

Transgressive segregation in both directions was observed for all traits for the three harvests in the annual × perennial ryegrass population (Table 1; Fig. 1). Most traits had intermediate broad sense heritabilities. Fiber components were positively correlated with each other and were individually negatively correlated with CP content. A total of 63 QTLs were detected for four traits across the three harvests from both the female and the male maps in the population. Coincident QTLs were identified for most traits measured from at least two harvests on both the female and the male maps. Consistent QTLs for different traits were also detected in the population.

We measured fiber components (NDF, ADF, ADL), and CP from 152 progeny individuals from the population as well as the MFA and MFB parents in three harvests varying by developmental stage or year. Phenotypic means of parents showed variation for most traits and fell within the range exhibited by progeny individuals, providing evidence for transgressive segregation in both directions (Table 1; Fig. 1). Although the parental values were in the same category group for ADF in the September 2003

Table 3 Putative QTL associated with fiber components and CP detected on female parental map by simple interval mapping in the annual × perennial ryegrass population

Trait[a]	Name of QTL[b]	Linkage group	LOD score	Position	Nearest marker	Maximum LOD position ± 1 unit (cM)	Phenotypic variance explained by QTL (%)
NDF	qNDF-Aug-03-f7	7	3.05	83	BCD938	68.3–83.9	32.1
	qNDF-Sep-03-f1	1	3.26	27	CDO98	25.7~33.9	13.4
	qNDF-Sep-03-f2	2	3.14	20.1	A-EaacMctt-245	10.1–23	21.4
	qNDF-Sep-03-f6	6	3.07	23.3	CDO1380	20.8–33.5	14.9
	qNDF-Sep-03-f7	7	3.8	60.1	G11.600	57.2–76.8	14.4
	qNDF-04-f1	1	4.0	88.1	CDO202	83.7–103.1	20.1
	qNDF-04-f3	3	4.24	61.3	P-EaccMcac-262	55.7–69.9	17.2
	qNDF-04-f5	5	3.10	0	E14.400	0–10	15.9
	qNDF-04-f7	7	3.17	69	CDO78	65.8–71.8	13.7
ADF	qADF-Aug-03-f1	1	3.19	79.2	CDO346	65.6–113.1	21.0
	qADF-Aug-03-f7	7	3.03	83	BCD938	68.3–83.9	29.5
	qADF-Sep-03-f1	1	3.16	25.1	BCD1072	19.3–33.9	15.1
	qADF-Sep-03-f7.1	7	3.55	41.5	TF69-188MFA	38.2–43.9	14.5
	qADF-Sep-03-f7.2	7	4.98	57.7	TF19-170	53.5–76.8	20.4
	qADF-04-f1.1	1	3.08	37	CDO278	30.4–54.2	12.0
	qADF-04-f1.2	1	4.78	88.1	CDO202	69.2–103.1	21.4
	qADF-04-f3.1	3	3.04	62.7	G7.450	50.3–73.6	12.5
	qADF-04-f3.2	3	3.36	83.3	A-EaagMcat-170	82.6–90.6	18.3
	qADF-04-f7	7	3.11	59	BCD782	57.7–71.8	10.1
ADL	qADL-Aug-03-f7	7	3.05	69	CDO78	57.7–76.8	13
CP	qCP-Aug-03-f1	1	3.81	74.2	K8.1800	58–88.1	26.8
	qCP-Aug-03-f2	2	3.44	87.3	E6.1550	85.3–116	17
	qCP-Sep-03-f2	2	3.07	48.2	F9.800	46.7–67	18.5
	qCP-Sep-03-f4	4	4.25	84.9	CDO795	58.3–92.6	17.8
	qCP-04-f2	2	3.22	57	CDO405	52–67	15.0
	qCP-04-f5	5	3.21	5	E14.400	0–12.6	18.8

[a] NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin; and CP, crude protein

[b] The QTL nomenclature followed the rules described by McCouch et al. (1997) in the form q-trait-year-female/male map LG. When there were more than one QTL for the same trait in the same map and same year, then different numbers were added as suffix at the end of QTL names to distinguish the QTLs

harvest and for CP in the August 2003 harvest (Fig. 1B, D), the progenies still exhibited substantial variation. This was because the parents of the mapping population could be heterozygous at most loci. The combination of different loci may cause tremendous variation when gametes are formed.

Most broad sense heritabilities for fiber components and CP are intermediate and some are low ($H_{ADF} = 0.35$ and $H_{ADL} = 0.46$ in the September harvest of 2003, Table 1), suggesting that MAS would be superior to conventional selection for decreasing fiber content. The heritabilities of most traits measured in the similar developmental stage were closer than that in different years. The heritabilities for the traits measured in August, whether in 2003 or 2004, were higher than that measured in September, 2003. That is probably due to different maturity of plants. In the August harvests, most plants were still in the vegetative growth stage, whereas in September, one month later, some plants had flowered already. Herbage quality is influenced greatly by reproductive development. Therefore, selection in the right developmental stage would be important for increasing digestibility in ryegrass breeding programs.

Phenotypic correlations were high between NDF and ADF in all harvests ($r = 0.86$, 0.72, and 0.82 for the three harvests, respectively; Table 2). This result agrees with the results reported by Cardinal et al. (2003) in forage maize. Consequently,

Table 4 Putative QTLs associated with fiber components and CP detected on male parental map by simple interval mapping in the annual × perennial ryegrass population

Trait[a]	Name of QTL[b]	Linkage group	LOD score	Position	Nearest marker	Maximum LOD position ± 1 unit	Phenotypic variance explained by QTL (%)
NDF	qNDF-Aug-03-m1	1	3.61	32.5	CDO105.2	29.9–33.1	24.3
	qNDF-Aug-03-m2	2	3.05	69.1	BCD1184	56.6–80.7	17.9
	qNDF-Aug-03-m6	6	4.16	90.2	TF41-204MFB	80.2–93.9	41.5
	qNDF-Sep-03-m1	1	3.64	79.6	CDO959	76.9–85	15.1
	qNDF-Sep-03-m6	6	3.27	90.2	TF41-204MFB	80.2–93.9	28.9
	qNDF-Sep-03-m7	7	3.98	51.6	A-EacgMcaa-352	49.8–63.9	16.6
	qNDF-04-m1	1	3.3	56.2	TF73-240MFB	55–57.1	12.4
	qNDF-04-m2	2	3.38	80.7	TF68-158MFB	76.9–90.7	14.9
	qNDF-04-m6	6	3.75	27.9	CDO400	0–36.9	15.6
	qNDF-04-m7	7	3.12	45.3	BCD782	43.1–53.3	14.7
ADF	qADF-Aug-03-m1	1	3.67	32.5	CDO	60.4–81.1	33.3
	qADF-Aug-03-m6	6	4.18	90.2	TF41-204MFB	80.2–93.9	47.7
	qADF-Sep-03-m1	1	3.49	79.6	CDO959	76.1–82.9	14.3
	qADF-Sep-03-m2	2	4.27	78.7	RZ69	76.9–94.2	23.1
	qADF-Sep-03-m7	7	3.11	51.6	A-EacgMcaa-352	49.8–64.4	11.1
	qADF-04-m1.1	1	3.54	56.2	TF73-240MFB	55–57.1	13.7
	qADF-04-m1.2	1	3.14	71.4	TF75-256MFB	67.6–72.9	10.9
	qADF-04-m2	2	3.05	78.7	RZ69	76.9–90.7	16.3
	qADF-04-m6	6	4.2	18.4	CDO534	0–39	19.5
	qADF-04-m7	7	3.45	45.8	A-EacgMcaa-277	44.2–53.3	16.4
ADL	qADL-Aug-03-m2	2	4.72	99.2	TF23-180MFB	94.2–100.6	45.0
	qADL-Aug-03-m6	6	3.48	90.2	TF41-204MFB	75.2–93.9	24.5
	qADL-Aug-03-m7	7	3.84	49.8	CDO78	48.9–60	15.2
	qADL-Sep-03-m2	2	3.13	0	CDO667	0–5	22.1
	qADL-Sep-03-m6	6	3.41	90.2	TF41-204MFB	80.2–93.9	37.1
	qADL-Sep-03-m7	7	3.33	60	BCD938	48.1–67.5	18.9
	qADL-04-m5	5	3.08	34.4	P-EagcMcat-221	19.2–38	14.1
	qADL-04-m6.1	6	3.07	27.9	CDO400	25.4–34.3	11.7
	qADL-04-m6.2	6	3.37	93.9	TF41-198	80.2–93.9	17.2
CP	qCP-Aug-03-m1	1	5.02	47.5	Fluorescence	45.2–49.2	22.1
	qCP-Aug-03-m2.1	2	3.82	42	A-EagcMcat-193	41.4–43.1	22.2
	qCP-Aug-03-m2.2	2	3.87	67.6	TF30-191MFB	59.3–76.6	21.4
	qCP-Sep-03-m2	2	4.66	21.2	A-EaccMcta-376	17.5–38.4	20.8
	qCP-Sep-03-m4	4	5.05	51.1	CDO795	43.1–70.1	19.7
	qCP-Sep-03-m7	7	3.23	57.9	A-EagcMctg-435	54.2–60	18.5
	qCP-04-m2	2	3.51	34.6	RZ395	21.2–38.4	14.1
	qCP-04-m5	5	3.78	13.4	*K9.1800	1.6–27.2	25.1

[a] NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin; and CP, crude protein

[b] The QTL nomenclature followed the rules described by McCouch et al. (1997) in the form q-trait-year-female/male map linkage group. When there were more than one QTL for the same trait in the same map and same year, then different numbers were added as suffix at the end of QTL names to distinguish the QTLs

selection for one trait would cause a correlated response of the other trait in the same population. Therefore, in ryegrass breeding programs, it may be only necessary to select for one of the two fiber components.

The QTL mapping of fiber components presented here is consistent with the high phenotypic correlations. The QTLs for NDF on LG 7, whether on the female or the male map, coincided with those for ADF (Tables 3, 4; Figs. 2, 3). The QTLs, qNDF-04-f1 on LG 1 and qNDF-04-f3 on LG3 on the female map, and the QTLs, qNDF-Sep-03-m1 on LG 1, qNDF-04-m2 on LG 2, and qNDF-Aug-03-m6 on LG 6 on the male map,

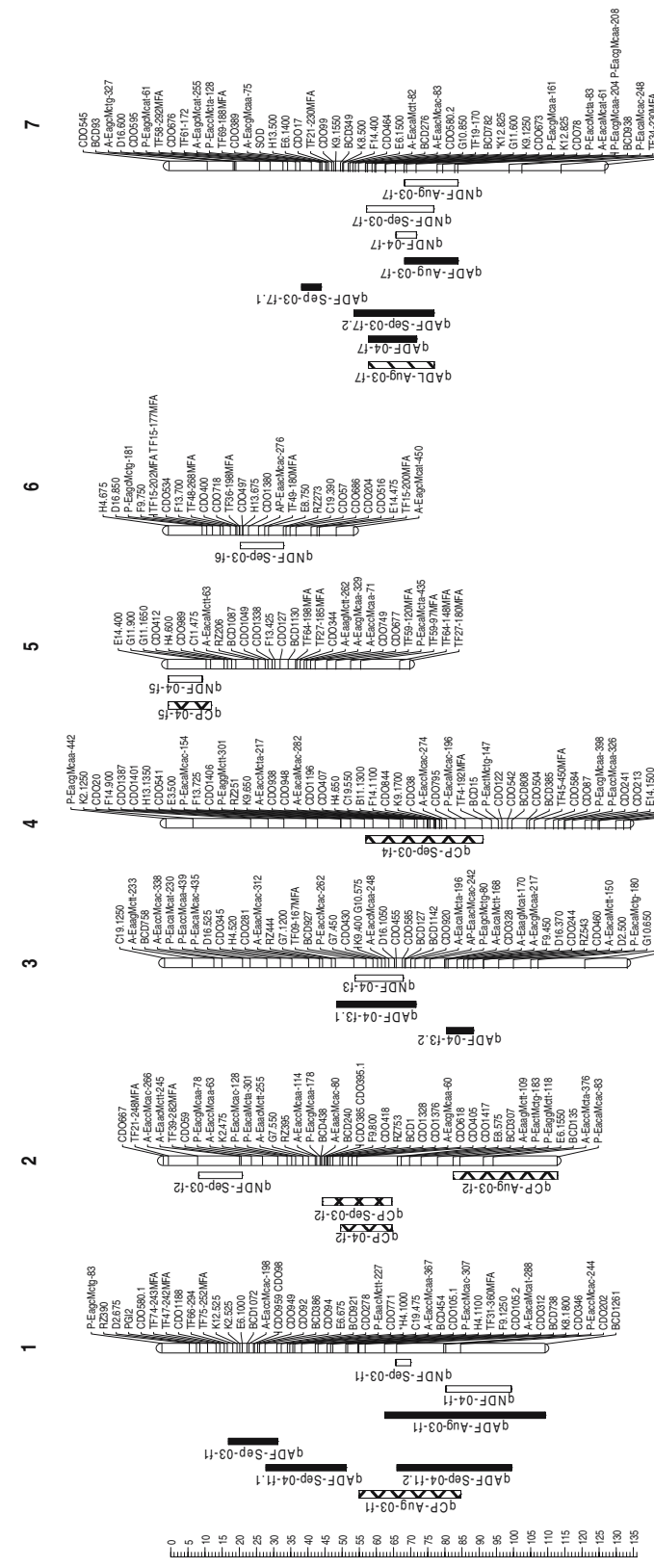

Fig. 2 Female parental map of the annual × perennial ryegrass interspecific hybrid showing QTLs for four traits for the three harvests over seven LGs. The QTLs for the four traits detected by simple interval mapping of MapQTL are given on the right side of each LG. The NDF was indicated by blank bars, ADF by black bars, ADL by hatched bars, and CP by stripped cross bars. Bar length represents a LOD drop of one unit from maximum likelihood position. The three harvests were taken in Aug., Sep., 2003, and Aug. 2004, respectively

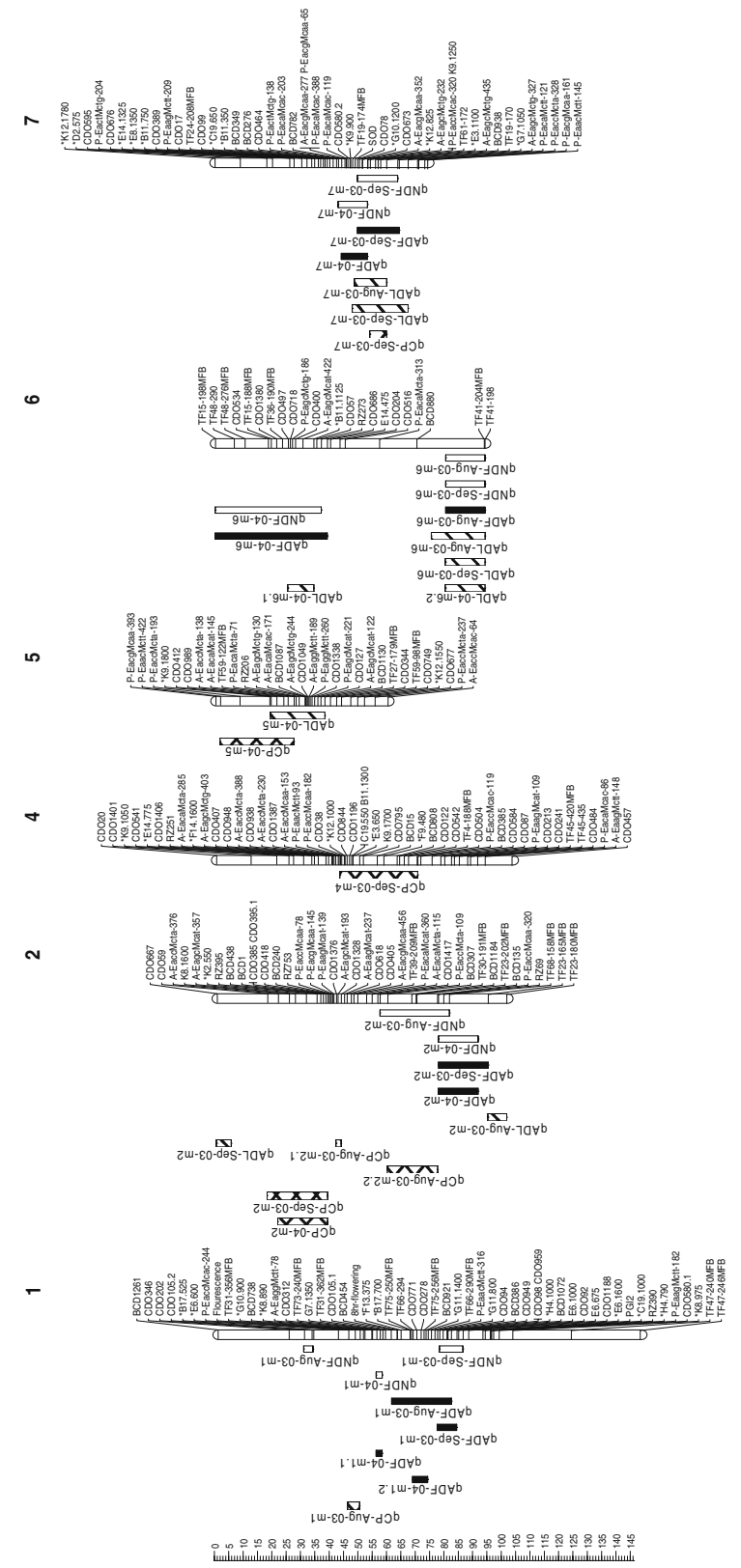

Fig. 3 Male parental map of the annual × perennial ryegrass interspecific hybrid showing QTLs for four traits for three harvests over seven LGs. The QTLs for the four traits detected by simple interval mapping of MapQTL are given on the right side of each LG. The NDF was indicated by blank bars, ADF by black bars, ADL by hatched bars, and CP by stripped cross bars. Bar length represents a LOD drop of one unit from maximum likelihood position. The three harvests were taken in Aug., Sep., 2003, and Aug. 2004, respectively

were co-localized with QTL for ADF. But trait specific QTL clusters were also found in this study. For example, the QTL on LG 2 near the marker A-EaacMctt-245 and the QTL on LG 6 near the marker CDO1380 on the female map were only associated with NDF, but not with ADF. Some QTLs for NDF and ADF also coincided in their genomic locations with QTLs for ADL. For example, the QTLs near the marker of CDO078 on LG 7 on both the female map and the male map (Tables 3, 4; Figs. 2, 3) affected all three fiber components. Another example was the QTL near the marker TF41-204MFB on the male map LG 6 (Table 4; Fig. 3) that was associated with NDF, ADF and ADL. These coincident QTLs provide evidence of the genetic basis of the observed high level of phenotypic correlation among the fiber components. The common QTL could be interpreted as a pleiotropic locus that controls the common sub-fraction of the fiber components, and therefore, would result in the concurrent increase or decrease of the correlated traits when we select for only one trait.

Consistent QTLs were also identified between fiber components and CP. For example, the QTL for CP detected on LG 7 of the male map in the September 2003 harvest (qCP-Sep-03-m7) was mapped to the similar regions for qNDF-Sep-03-m7, qADF-Sep-03-m7, and qADL-Sep-03-m7 (Table 4; Fig. 3). The QTL for CP on LG 1 on the female map, qCP-Aug-03-f1, was another example for the consistent QTL with NDF, qADF-Aug-03-f1 (Table 3; Fig.3). The negative correlation between fibers and protein could come from those common QTLs with opposing effects. Thus, we may reach the breeding goal of decreasing the fiber components and increasing CP simultaneously through selection for either of the traits.

Most genes for cellulose or hemicellulose biosynthesis in ryegrass are currently unknown. However, a few genes for the lignin biosynthesis pathway, such as *Lp*CCR1, *Lp*OMT1 and *Lp*CAD2, have been identified (Heath et al. 1998; Lynch et al. 2002; McInnes et al. 2002) and were mapped at RFLPs to LG 7 and LG 2 in the ryegrass P150/112 genetic map (Cogan et al. 2005). In addition, the *Lp*CCR1 gene was mapped as a single nucleotide polymorphism (SNP) locus to a coincident location on the F1 (NA6 × AU6) genetic map (Faville et al. 2004), along with a SNP markers for a number of unannotated *Lp*CAD-like genes which are co-regulated with the *Lp*CAD1 and *Lp*CAD3 genes (Cogan et al. 2006). In our study, the majority of the QTLs for NDF, ADF, and ADL were primarily detected on these two linkage groups (LG 2 and LG 7). Moreover, coincident QTLs for NDF and ADF were also found on LG 6 in the present study. Although the lignin biosynthetic genes of perennial ryegrass (*Lp*CCR1, *Lp*OMT1 and *Lp*CAD2) were not mapped to LG 6, their ortholoci from wheat EST were mapped to wheat chromosome 6L (Cogan et al. 2005). Comparative mapping indicates that wheat 6L is the syntenic counterpart of the corresponding region of ryegrass LG 6 (Jones et al. 2002; Sim et al. 2005). This suggests that other members of lignin biosynthesis gene family may be located on LG 6 (Cogan et al. 2005).

The QTLs identified for fiber components and CP from our study are largely consistent but vary in a few respects from the results reported by Cogan et al. (2005) in another ryegrass population. The QTLs for NDF and Est ME (related to ADF) on LG 7 mapped by Cogan et al. (2005) coincided with the QTLs for NDF and ADF in our study. However, we did not find coincident QTLs on LGs 3 and 5 for NDF that was reported by Cogan et al. (2005). We found only one individual QTL on LG 3 and LG 5, respectively on the female map from the 2004 harvest. Cogan et al. (2005) also reported that the coincident QTLs for CP were located on LG3, but only individual QTLs were detected in a single experimental dataset on LG 1, 2, 4 and 5. In our study, we detected coincident QTLs for CP on LG 2, individual QTLs from a single harvest were distributed on LGs 1, 2, 4, 5, and 7. The discrepancy could result from the difference in the mapping populations used for mapping. The population in the present study is an interspecific ryegrass hybrid, which is likely to maximize differences for a range of phenotypic characters. Lübberstedt et al. (1997 reported that QTLs related to some forage quality were not consistent among different forage maize populations.

Many QTLs for NDF, ADF, ADL and CP were identified in forage maize (Lübberstedt et al. 1997, 1998; Mechin et al. 2001; Cardinal et al. 2003) and some QTLs are known to link to

the genes involved in cellulose and lignin biosynthesis (Cardinal et al. 2003). Although the lower resolution of comparative genetic mapping analysis between maize and ryegrass compared to the Triticeae cereals limits their comparison of genetic region, the phenotypic correlation between NDF and ADF and QTL clustering for the traits are similar with the two species.

Although many coincident QTLs were identified in the current study, we also detected a number of variable QTLs for the same trait in different harvests, suggesting the presence of QTL × environment variation. The interaction of QTLs and environment were observed in many studies (Lu et al. 1996; Yadav et al. 2003; Cogan et al. 2005). Temperatures and plant maturity also affect the fiber values measured. High temperatures often cause high fiber content thus low forage quality. The magnitude of variations might be different in different fiber components, which causes the inconsistency of the QTL detection. In addition, different magnitude of errors in different experiments may have also contributed to the inconsistency. Coincident QTLs provide relative stability of genetic control, which would overcome the problem associated with the interaction of QTL and environment.

In our study, groups of coincident QTLs were identified on LGs 2, 6 and 7 for NDF, LGs 1, 2 and 7 for ADF, LGs 6 and 7 for ADL, and LG 2 for CP, respectively. The QTLs on LGs 2, 6 and 7 identified for fiber components were co-located on the same LG with several lignin biosynthetic genes from perennial ryegrass. The identification of QTLs affecting those fiber components and CP provides new knowledge about the genetics of digestibility of ryegrass and the functionally associated markers related to those QTLs will serve as a useful tool for MAS for high digestibility of ryegrass. The QTL clustering will give information on involved mechanisms and genes of cell-wall digestibility.

Acknowledgements We wish to thank Dr. Mike Casler for critically reading the manuscript. We also express our appreciation to Trish Patrick for technical assistance in laboratory analyses of forage quality traits, and to Lori Evans for technical assistance in maintaining the plants in the greenhouse.

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